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Evaluation of Antioxidant, Antibacterial, and Antifungal Properties of *Satureja hortensis* Essential Oil

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Background: *Satureja* is a genus belonging to the aromatic plants of Lamiaceae family. The genus *Satureja L.* (*Lamiaceae*) comprises more than 30 species of aromatic herbs and shrubs, widely distributed over the Mediterranean region. The genus is represented by 14 species in Iran of which, eight are endemic. Many species of the genus *Satureja* are reported to have aromatic and medicinal properties. The leaves, flowers, and stems of this plant are used as herbal tea and in treatment of various ailments in traditional medicine.
Objectives: The aim of this study was to investigate antimicrobial and antioxidant activity of essential oil (EO) of *Satureja hortensis* (*Lamiaceae*) that grows in Sabalan Mountain (Ardebil province, Iran).
Materials and Methods: This EO was tested in vitro against two bacterial species by disk and agar well diffusion methods and against four bacterial species and three *Candida* strains by broth microdilution method. Total phenol, flavonoid, and free radical scavenging activity of EO were evaluated.
Results: Total phenolic content was determined by using Folin-Ciocalteu reagent (32.65 mg/g), which was presented as gallic acid equivalent in 1 g of sample. IC50 of EO and ascorbic acid in DPPH method were respectively 277.9 and 19.34 μg. Minimum inhibitory concentration of the EO of *S. hortensis* against *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli,* and *Bacillus cereus* was respectively 2.5%, 2.5%, 5%, and 2.5%. The inhibition zone of EO in disk and agar well diffusion method showed that inhibitory zone on *B. cereus* was higher than that on *S. typhimurium* in both methods and *B. cereus* was more sensitive to EO. MIC to minimal fungicidal concentration (MFC) ratio of *S. hortensis* EO against *Candida albicans*, *Candida parapsilosis,* and *Candida krusei* in broth microdilution method were respectively 0.048%:0.048%, 0.024%:0.024%, and 0.012%:0.012%.
Conclusions: The data of the study clearly indicated that the EO of *S. hortensis* has a strong antioxidant, antibacterial, and antifungal activity.

Keywords: Essential Oils; Antioxidants; Traditional Medicine

1. Background

Satureja is a genus belonging to the aromatic plants of Lamiaceae family. The genus *Satureja L.* (*Lamiaceae*) comprises more than 30 species of aromatic herbs and shrubs, widely distributed over the Mediterranean region. These are annual aromatic plants that grow in arid, sunny, rocky habitats (1). The genus is represented by 14 species in Iran of which, eight are endemic. These aromatic species are mainly found in the north, northwestern, and western parts of Iran (2). Many species of the genus *Satureja* are reported to have aromatic and medicinal properties. The aerial parts of these species have distinctive tastes and can be added to stuffing and sausages. The leaves, flowers, and stems are used as herbal tea and, in traditional medicine, to treat various ailments such as cramps, muscle pains, nausea, and infectious diseases (3). In earlier investigations, *Satureja* species have been studied with respect to EO composition and have shown to be rich in components such as carvacrol, γ-terpinene, thymol, and p-cymene (4, 5).

2. Objectives

We aimed to investigate the antioxidant, antibacterial, and antifungal activities of the isolated EO of *Satureja hortensis* collected from Sabalan Mountain, Ardebil province, Iran.

3. Materials and Methods

3.1. Plant Material and Preparation of Essential Oil

The aerial part of *S. hortensis* was collected from Sabalan Mountain, Ardebil, Iran. Air drying of the plant was performed in a shady place for 14 days at room temperature.

The plants were verified in Botanical Group of Tehran Medicinal Herbs Research Centre, Tehran University of Medical Science. Plant was extracted via distillation by water for three hours using a Clevenger unit. The EO was dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4°C until further evaluations.

3.2. Flavonoid Contents

Flavonoid contents were estimated according to aluminum chloride colorimetric method. One milliliter of diluted EO of *S. hortensis* was mixed with 1 mL of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 minutes, the absorbance was measured at 430 nm. Total flavonoids were expressed through the calibration curve of Standard. All samples were analyzed in three replications (6).

3.3. Assay for Total Phenolic Content

Total phenolic constituent of EO of *S. hortensis* was performed employing the literature methods (7, 8) using Folin-Ciocalteu reagent and gallic acid (both Sigma-Aldrich) as standard. Briefly, 1 mL of various concentration of EO was mixed with Folin-Ciocalteu reagent (5 mL, 1:10 diluted with nanopure water) and incubated in room temperature. After ten minutes, 4 mL of 7.5% Na_2CO_3 solution was added and the mixture was allowed to stand for 30 minutes with intermittent shaking. Absorbance was measured at 765 nm. The same procedure was repeated for gallic acid and a standard curve was obtained.

3.4. Determination Antioxidant Activity (DPPH Assay)

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a free radical generating compound which is used to determine the radical scavenging activity of extracts and EOs (9). The DPPH radical scavenging assay has been used widely to evaluate the radical scavenging activity of the different type of antioxidant substances (10). The color changes will allow the detection of the scavenging activity at 517 nm. The half-maximal inhibitory concentration (IC_{50}) is often used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. Aliquots (50 μL) of various concentrations of the EO were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in following way:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The antioxidant activity of the extract was expressed as IC_{50} (11, 12). Ascorbic acid was used as the positive control and all tests were performed in triplicate.

3.5. Tested Microorganisms

Antibacterial activity of EO of *S. hortensis* was investigated against two gram-negative bacteria (*S. typhimurium* ATCC 13311, *E. coli* ATCC 43894) and two gram-positive bacteria (*B. cereus* ATCC 11178, *L. monocytogenes 19118*). This EO also was tested against three fungal strains including *Candida albicans* ATCC 10231, *Candida krusei 22113,* and *Candida parapsilosis* ATCC 22019.

3.6. Antimicrobial Bioassay

The in vitro antibacterial activity of EO was performed by disc diffusion, agar well diffusion, and broth microdilution methods. Antifungal activity of EO was evaluated by broth microdilution assay. Briefly, 200 μL of EO was diluted in 1 mL of 10% DMSO. Then serial dilution of EO of *S. hortensis* was performed.

3.7. Agar Well Diffusion Test

The used agar well technique was based on the well-established method of Deans and Ritchie (1987) with slight modifications. The freshly prepared inoculums (10^8 CFU/mL) was swabbed all over the surface of the nutrient agar plate (Merck, Germany) using sterile cotton swab. Four wells were bored in the medium with the help of sterile cork-borer having 6-mm diameter and were labeled properly. Then 35 μL of serially diluted EO were added to the wells. Plates were incubated at 37°C for 24 hours. After incubation, plates were observed for zone of inhibition (13).

3.8. Disk Diffusion Test

This method is presented as a consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS) (2001). EOs were diluted in 10% DMSO to the test concentration (20%, 10%, 5%, and 2.5%). Antimicrobial tests were performed by disc diffusion method using 100 μL of suspension containing 2×10^8 CFU/mL of bacteria. The discs (6 mm in diameter) were impregnated with 35 μL of the diluted oil and placed on the inoculated agar. Negative controls were prepared using the same solvents to dissolve the EO. The inoculated plates were kept at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms.

3.9. Broth Microdilution Assay

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the NCCLS (2003). The inocula of the bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The 10% DMSO was used to dissolve the EO and then diluted to the highest concentration (200 μL/mL). A serial doubling dilution of the oil was prepared in a 96 well mi-

croliter plate in inoculated nutrient broth (the final concentration in each well was adjusted to 2×10^6 CFU/mL). The plate was incubated for 24 hours at 37°C.

3.10. Determination of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration

For determination MIC, microdilution broth method was used based on the Clinical Laboratory Standards Institute (CLSI, M27-A2). Stocks and dilutions of EO were prepared in 10% DMSO. Final concentrations in the microdilution plates ranged from 12.5% to 0.012% (v/v). The microdilution plates were prepared by using the RPMI 1640 broth medium (Sigma) with L-glutamine and without sodium bicarbonate and buffered at pH of 7.0 with 0.165 mol/L of morpholinepropansulfonic acid (MOPS) (Sigma). Yeast suspensions were prepared after Shacking and adjusting to a 0.5 McFarland standard transmittance at a wavelength of 530 nm. The final yielded inoculum was of 0.5×10^3 to 2.5×10^3 cells/mL. Two wells served as the growth control and sterility check. MICs were visually determined at 24 hours of incubation at 35°C, and were observed for the presence or absence of growth. The growth in each well was compared with that of the growth control EO-free well (14).

4. Results

The oil isolated by hydrodistillation from the aerial part of *S. hortensis* was found to be yellow liquid and was obtained in yields of 0.9% (v/w).

4.1. Total Phenolic and Flavonoid Contents

The total phenols content, which were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 29.857x + 0.0437$; $R^2 = 0.9968$), was 32.65 mg/g, presented as gallic acid equivalent in mg/g EO.

4.2. DPPH Assay

Free radical scavenging activity of EO of *S. hortensis* was measured by DPPH assay. Free radical scavenging increased by increasing EO concentration. The concentration of EO of *S. hortensis* resulting in 50% inhibition of the free radical (IC50) is shown in Figure 1. The IC50 level of EO of *S. hortensis* was higher than standard.

4.3. Disk Diffusion and Agar Well Diffusion Methods

The antimicrobial activity of EO of *S. hortensis* against microorganisms was examined in the present study and its potency was assessed by the presence or absence of inhibition zone and MIC values. The results are given in Table 1. The data of the study clearly indicated that the EO has strong antibacterial and antifungal activities.

4.4. Broth Microdilution Assay

The data of the study clearly indicated that the EO of *Satureja* had antibacterial activity against a number of bacteria. MIC of EO of *S. hortensis* against *S. typhimurium*, *L. monocytogenes*, *E. coli*, and *B. cereus* was respectively 2.5%, 2.5%, 5%, and 2.5%.

4.5. Antifungal Assay

Table 2 illustrates the inhibitory activity of EO on the growth of different *Candida* species including *C. albicans*, *C. parapsilosis,* and *C. krusei*. As shown in table, the lower MIC was related to *C. krusei* with MIC of 0.012 µL/mL.

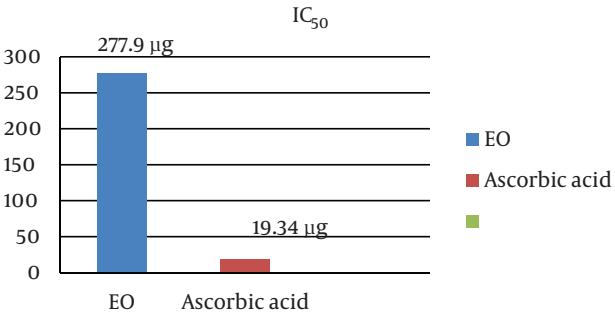

Figure 1. The Concentration of Satureja Essential Oil and Ascorbic Acid Resulting in 50% Inhibition of the Free Radical

Table 1. Inhibition Zone of Different Concentrations of *Satureja hortensis* Essential Oil on Bacterial Strains in Disk and Agar Well Diffusion Method [a]

Microorganism	Concentrations of *Satureja hortensis* Essential Oil			
	20%	10%	5%	2.5%
Disk diffusion				
Salmonella typhimurium	25	24	20	15
Bacillus cereus	34	22	20	17
Agar well				
Salmonella typhimurium	25	22	19	15
Bacillus cereus	30	26	20	15

[a] Data are presented in millimeter.

Table 2. Antifungal Testing of *Satureja hortensis* Essential Oil by Broth Microdilution Method [a,b]

Microorganisms	MIC	MFC
Candida albicans	0.048	0.048
Candida krusei	0.012	0.012
Candida parapsilosis	0.024	0.024

[a] Abbreviations: MIC, minimum inhibitory concentration; and MFC, minimum fungicidal concentration.
[b] Data are presented as %.

5. Discussion

Plant's EOs are a potentially useful source of antimicrobial compounds. EOs are natural products extracted from vegetal materials that can be used as natural additives in many foods because of their antibacterial, antifungal, antioxidant, and anticarcinogenic properties (15). The data obtained from the disc and agar well diffusion methods indicated that the EO displayed a variable degree of antimicrobial activity on tested strains. The data indicated that gram-negative *S. typhimurium* was more resistant to the EO and has smaller inhibition zone. Skocibusic et al. reported that inhibition zone of EO of *S. hortensis* on *B. cereus* at 10 and 20 μL per disk was 14 and 23 mm, respectively. Similar to our study, The inhibitory effect increased by increasing oil concentration (16). The results of MIC indicate that the oil exhibited varying levels of antimicrobial activity against the investigated food pathogens in a range of 2.5% to 5%. The higher level of MIC was related to the *E. coli*. Skocibusic et al. reported that *S. typhimurium* was more resistant to EO than *E. coli* and *B. cereus* were (16). In previous studies, the EO of *S. hortensis* L. showed antifungal activity against phytopathogenic (17) and food spoilage fungi (18). The major component of this oil, carvacrol, is capable of inhibiting aflatoxin production by *Aspergillus parasiticus* (19) and *Aspergillus flavus* in a liquid medium and tomato paste (20, 21). The same authors suggested that carvacrol could be useful in controlling aflatoxin contamination of susceptible crops. Adiguzel et al. reported that the oil of *S. hortensis* exhibited high antifungal activity against *Aspergillus niger* (MIC/minimal bactericidal concentration [MBC], 0.78 μL/mL), *Saccharomyces cerevisiae* (MIC/MBC, 0.39/0.20 μL/mL), and *C. albicans* (MIC/MBC, 0.20 μL/mL). The oil from Turkey did not show any effect against *C. albicans* (18). According to our results, EO of *S. hortensis* collected in Sabalan Mountain showed higher activity in comparison to the results from previous studies. The EO of *S. hortensis* can be considered as a useful natural agent for the treatment of candidiasis. The antioxidant activity of the EO of *S. hortensis* indicates that the EO has a protective effect against radical oxygen species and therefore, it can be used as a natural preservative ingredient in the food or pharmaceutical industry.

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References

1. Slavkovska V, Jancic R, Bojovic S, Milosavljevic S, Djokovic D. Variability of essential oils of Satureja montana L. and Satureja kitaibelii wierzb. ex Heuff. from the central part of the balkan peninsula. *Phytochemistry.* 2001;**57**(1):71–6.
2. Jamzad Z. Satureja rechingeri (Labiatae)—a new species from Iran. *Ann Nat Mus Wien.* 1996;**98**:75–7.
3. Amiri H. The in vitro antioxidative properties of the essential oils and methanol extracts of Satureja macrosiphonia Bornm. *Nat Prod Res.* 2011;**25**(3):232–43.
4. Konakchiev A, Tsankova E. The Essential Oils of Satureja montana ssp. kitaibelii Wierzb. and Satureja pilosa var. pilosa Velen from Bulgaria. *J Essent Oil Res.* 2002;**14**(2):120–1.
5. Sajjadi SE, Baluchi M. Chemical composition of the essential oil of Satureja boissieri Hausskn. ex Boiss. *J Essent Oil Res.* 2002;**14**(1):49–50.
6. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 2006;**97**(4):654–60.
7. Chandler SF, Dodds JH. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of solanum laciniatum. *Plant Cell Rep.* 1983;**2**(4):205–8.
8. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic.* 1977;**28**(1):49–55.
9. Barros L, Ferreira MJ, Queiros B, Ferreira I, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food chem.* 2007;**103**(2):413–9.
10. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxy-flavones. *Free Radic Biol Med.* 1996;**20**(1):35–43.
11. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res.* 2000;**14**(5):323–8.
12. Cuendet M, Hostettmann K, Potterat O, Dyatmiko W. Iridoid Glucosides with Free Radical Scavenging Properties fromFagraea blumei. *Helv Chim Acta.* 1997;**80**(4):1144–52.
13. Deans SG, Ritchie G. Antibacterial properties of plant essential oils. *Int J Food Microbiol.* 1987;**5**(2):165–80.
14. *Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-18.* .Wayne, PA: Clinical and Laboratory Standards Institute.; 2008.
15. Teissedre PL, Waterhouse AL. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *J Agric Food Chem.* 2000;**48**(9):3801–5.
16. Skocibusic M, Bezic N, Dunkic V. Phytochemical composition and antimicrobial activities of the essential oils from Vis. growing in Croatia. *Food Chemistry.* 2006;**96**(1):20–8.
17. Boyraz N, Ozcan M. Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (Satureja hortensis L.) growing wild in Turkey. *Int J Food Microbiol.* 2006;**107**(3):238–42.
18. Adiguzel A, Ozer H, KiliC H, CetiN B. Screening of antimicrobial activity of essential oil and methanol extract of Satureja hortensis on foodborne bacteria and fungi. *Czech J Food sci.* 2007;**25**(2):81–9.
19. Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Jaimand K, Nagasawa H, et al. Inhibitory effects of Satureja hortensis L. essential oil on growth and aflatoxin production by Aspergillus parasiticus. *Int J Food Microbiol.* 2008;**123**(3):228–33.
20. Omidbeygi M, Barzegar M, Hamidi Z, Naghdibadi H. Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. *Food Control.* 2007;**18**(12):1518–23.
21. Dikbas N, Kotan R, Dadasoglu F, Sahin F. Control of Aspergillus flavus with essential oil and methanol extract of Satureja hortensis. *Int J Food Microbiol.* 2008;**124**(2):179–82.